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| 09/937,137 | 09/21/2001 | Giammaria Sitar | 1271-001 | 4515 |

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| EXAMINER | |
|---|---|
| AFREMOVA, VERA | |
| **ART UNIT** | **PAPER NUMBER** |
| 1651 | |

DATE MAILED: 11/24/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/937,137 | SITAR, GIAMMARIA | |
| | Examiner | Art Unit | |
| | Vera Afremova | 1651 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *16 September 2004 and 30 June 2004*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1,3-21 and 24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-21 and 24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

## DETAILED ACTION

Claims 1, 3-21 and 24 {supplemental amendment 9/16/2004 and amendment 6/30/2004} are pending and under examination.

Claims 2, 22 and 23 were canceled by applicant.

### *Claim Rejections - 35 USC § 112*

Claims 1, 3-21 and 24 as amended and new are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

1. Claims 1, 11 and 24 are indefinite because it is unclear as claimed what is a final product. It is uncertain as claimed when and what blood cells or blood cell fractions are physically separated (isolated) from each other.

a. The claimed invention is not consistent in identification of "fetal NRBCs" and "NRBCs" derived from maternal blood, for example: see claim 1, lines 1, 19, 21 and 21; and, for example: see claim 11 and claim 17, wherein terms "fetal" or "said fetal" appear to lack antecedent basis. It is not clear from the record that these two entities {"fetal NRBCs" and "NRBCs"} are identical. For example: see specification (page 2, lines 1- 15, page 10, line 19) wherein it is disclosed that stem cells might be present in adult blood.

b. The applicant's method as disclosed demonstrates that centrifugation step results in a possession of a cell fraction that contains fetal nucleated red blood cells (NRBCs) together with maternal nucleated lymphocytes and monocytes (specification page 10, lines 16-19; page 4, line 12). However, the term "isolated" NRBCs in claim 1 appears to encompasses physical separation of fetal NRBCs from all other cells (see steps c, d and e). Claim 13 says that "maternal blood

cells are lymphocytes and monocytes" and, thus, it appears that claimed invention encompasses physical separation of maternal monocytes from fetal nucleated cells.

Thus, meaning of cellular terms in the claimed invention is uncertain in the light of specification. Therefore, it is unclear what is a final product in the claimed method.

2. Claims 10, 16, 20 and 21 are unclear about meaning of "a single separation device" and/or "a single centrifugation step" because the issue of cells that are separated/isolated is uncertain as claimed and as intended.

***Claim Rejections - 35 USC § 102***

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

1. Claims 11-17, 21 and 24 are rejected under 35 U.S.C. 102(b) as being anticipated by Boyer et al. {Blood. June 1976. Vol. 47, No. 6, pages 883-897}.

Claims are directed to a method for separating and identifying fetal nucleated red blood cells (NRBCs) present in maternal blood wherein the method comprises combining a sample of maternal peripheral blood with a tissue culture medium to form a non-physiological mixture having pH 6.4-6.6, subjecting the mixture to centrifugation in a discontinuous density gradient causing isolation/separation of NRBCs and further washing, resuspending and ascertaining the presence of fetal NRBCs. Some claims are further drawn to the use of Ficoll during centrifugation, to the use of a single separation device and/or single centrifugation step.

The reference by Boyer et al. {Blood. June 1976. Vol. 47, No. 6, pages 883-897} discloses a method for enrichment of fetal red cells or erythrocytes of fetal origin (that are fetal NRBCs) from adult blood or peripheral maternal blood via selective hemolysis of adult blood cells and centrifugation. The method comprises combining a sample of maternal blood cell with a medium or solution to form a non-physiological mixture having pH 6.5-6.6 (page 885, line 7), subjecting the mixture to centrifugation in a discontinuous density using Ficoll (page 885, par. 2). The fraction of cells of with fetal NRBCs is separated, washed, resuspended (page 885, section "harvest"). The presence of red cells of fetal origin is ascertained (page 895, par. 1). Thus, the cited reference appears to disclose the same method for separating and identifying fetal red blood cells (NRBCs) as claimed because the cited method comprises same steps and same elements as claimed within the meaning of the instant claims.

The same active steps are steps of making non-physiological mixture, separating cell fractions by centrifugation, washing, resuspending, ascertaining presence of fetal cells in separated cell fraction. The same elements are maternal peripheral blood as starting cell sample, identical pH 6.5-6.6 of a non-physiological mixture, same agent Ficoll during centrifugation. Although the cited reference appears to teach several centrifugation repetitions, the claimed method is not particularly clear what is encompassed by "a single separation device" and/or "a single centrifugation step" and/or what cell fractions are separated from each other as intended.

2. All presently pending claims as amended and new remain/are are rejected under 35 U.S.C. 102(a) as being anticipated by Sitar et al. {Cytometry, April 1, 1999. Vol. 35, No. 4, pages 337-345} for the same reasons as explained in the prior office action. However, this

rejection will be withdrawn upon filing corrected copy of Declaration by Giammaria Sitar as discussed and proposed by applicant(s) during interview 9/15/2004. The proposed draft establishes what role non-inventors co-authors played in the publication.

***Claim Rejections - 35 USC § 103***

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 1 and 3-5, 7 and 8 remain rejected under 35 U.S.C. 103(a) as being unpatentable over US 5,641,628 [D] taken with US 5,676,849 [B], US 5,432,054 [C] and Guyton [U] as explained in the prior office action and repeated herein.

Claims are directed to a method for isolating or preparing fetal nucleated red blood cells (NRBCs) present in maternal peripheral blood for prenatal genetic investigation wherein the method comprises step of combining a maternal blood sample with a tissue culture medium and an aqueous solution comprising citrate in order to form a mixture having specific characteristics that are pH 6.4-6.6, osmolarity 300-330 mOsm, $Na^+$ 150-160 mmol/l, $K^+$ 4.5-5.5 mmol/l, $Cl^-$ 100-115 mmol/l, $Ca^{++}$ 1.00 –2.50 mmol/l, glucose 400-500 mg/dl, lactate 10-20 mg/dl; step of transferring the mixture to a cell separation device and adding a high density liquid containing a red blood cell aggregating agent, step of isolating NRBCs by subjecting the separation device to centrifugal force, step of washing and resuspending the isolated NRBCs and step of identifying and counting fetal NRBCs. Some claims are further drawn to the use of a liquid containing a red blood cells aggregating agent such as Ficoll containing preparation. Some claims are further drawn to the use of a liquid in separation device with 1.068 g/ml density. Some claims are

further drawn to the use a cell separation device or apparatus in a method for isolating fetal cells present in maternal peripheral blood for prenatal genetic investigation.

The cited patent US 5,641,628 [D] is relied in the instant office action for the disclosure of methods for isolating nucleated fetal cells from maternal peripheral blood intended for prenatal genetic investigation (example 10) wherein the method encompasses isolation of fetal nucleated red blood cells by density gradient centrifugation of maternal blood which has been modified by addition of citrate dextrose aqueous solution (col. 22, line 42). The cited patent also teaches steps of transferring the mixture to a cell separation device, adding a high density liquid with Ficoll, step of isolating mononuclear cells including NRBCs by subjecting the separation device to centrifugal force, step of washing and resuspending the isolated cells and step of identifying fetal NRBCs with antibodies to precursors of hematopoietic cells and by PCR techniques with Y chromosome primers.

The cited patent US 5,641,628 [D] is silent with regard to the final characteristics of the modified blood sample. However, the claimed amounts for $Cl^-$, $Ca^{++}$, and lactate are the same as in normal blood sample as evidenced by Guyton (page 277). Thus, the blood sample of the cited patent US 5,641,628 provides for the same amounts for $Cl^-$, $Ca^{++}$, and lactate in the resulting blood mixture as encompassed by the claimed invention. The reference by Guyton also demonstrates that osmolarity of normal blood is about 302 mOsm that is within the presently claimed ranges. Thus, the blood sample of the cited patent US 5,641,628 provides for the same osmolarity as encompassed by the claimed invention. Further, osmolarity in the method of the cited patent US 5,641,628 [D] is reasonably expected to be increased after addition of the aqueous citrate-dextrose solution. The presently claimed amounts for $Na^+$ and $K^+$ are about the

same or slightly higher than in normal blood according to the reference by Guyton. But the citrate aqueous solution of the cited patent US 5,641,628 [D] is likely to provide for additional sodium and/or potassium. The cited patent US 5,641,628 [D] also suggests that the blood sample is stored overnight with the culture medium RPMI (col. 13, line 42) and, thus, the blood sample is reasonably expected to contain about the same amounts of potassium and/or sodium as encompassed by the claimed invention. The cited patent US 5,641,628 is silent about pH value. But the cited patent US 5,676,849 [B] demonstrates that the commonly used citrate-dextrose/glucose aqueous solution contain citric acid (col. 6, line 58 or col. 10, lines 39). Thus, the solution in the method of US 5,641,628 [D] is reasonably expected to provide for pH low that neutral in the method for fetal cells preparation or identification.

The cited patent US 5,641,628 [D] teaches the separation of cells by liquid density gradient centrifugation but it is silent about the liquid density in the method for separation of fetal cells from maternal blood. However, the cited patent US 5,432,054 [C] teaches the use of liquid density gradient centrifugation for separation of fetal cells from maternal blood wherein the liquid density gradient includes 1.065 g/ml (col. 12, table 2) or about 1.068 g/ml for centrifugation of modified maternal blood as encompassed by the presently claimed method.

Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to practice a method for isolating fetal cells present in maternal peripheral blood for prenatal genetic investigation wherein the method encompasses isolation of fetal nucleated red blood cells by density gradient centrifugation of maternal blood modified by addition of acid-citrate-dextrose (glucose) preparation as taught and suggested by the cited patents US 5,641,628 [D] and US 5,676,849 [B] with a reasonable expectation of success in

isolating fetal nucleated red blood cells as demonstrated by the cited patents. The concept of isolating fetal cells from maternal blood of the cited patents US 5,641,628 [D], US 5,676,849 [B] and US 5,432,054 [C] is based on a density gradient centrifugation isolation of fetal nucleated red blood cells from modified maternal blood and it is similar to the concept of the presently claimed method which is also based on a density gradient centrifugation isolation of fetal nucleated red blood cells from modified maternal blood. The characteristics of the resulting modified blood sample that are claimed appear to be about the same as encompassed by the cited US 5,641,628 [D] as evidenced by Guyton. Although the cited patent(s) are lacking the particular disclosure about dextran, the final characteristics of the modified blood sample that are claimed are not affected by dextran whether it is added or not to the blood sample. Thus, the claimed invention as a whole was clearly prima facie obvious, especially in the absence of evidence to the contrary. One of skill in the art would have been motivated to used acid-citrate-dextrose solution for the expected benefits in blood cell separation because addition of this aqueous solution is a common practice in the methods for separation of fetal cells from maternal blood as adequately demonstrated by the cited US 5,641,628 [D] and US 5,676,849 [B]. Thus, the claimed subject matter fails to patentably distinguish over the state art as represented be the cited references.

Therefore, the claims are properly rejected under 35 USC § 103.

Claims 1, 3-10 remain/are rejected under 35 U.S.C. 103(a) as being unpatentable over US 5,641,628 [D] taken with US 5,676,849 [B], US 5,432,054 [C] and Guyton [U] as applied to

claims 1, 3-5, 7 and 8 above, and further in view of US 4,424,132 [D], GB 2-75376 [N] and FR 77 08053 [O] as explained in the prior office action and repeated herein.

Claims 1, 3-5, 7 and 8 as explained above. Claims 6, 9 and 10 are further drawn to the use of a cell separation device or apparatus with elongated chamber and channel(s) that are open to the chamber in a method for isolating fetal cells present in maternal peripheral blood for prenatal genetic investigation.

The cited patents US 5,641,628 [D], US 5,676,849 [B] and US 5,432,054 [C] are relied upon as explained above for the disclosure of methods for isolating nucleated fetal cells from maternal peripheral blood intended for prenatal genetic investigation wherein the methods encompass isolation of fetal nucleated red blood cells by density gradient centrifugation of modified maternal blood in various cell separation devices. The cited patents are silent about design of cell separation devices. However, the methods of the cited patents encompass the use of generic cell separation devices and they result in successful separation of fetal cells. Thus, there is a reasonably believe that the cell separation devices of the cited patents are suitable and appropriate in the methods for isolating nucleated fetal cells from maternal peripheral blood intended for prenatal genetic investigation.

Additional references US 4,424,132 [D], GB 2-75376 [N] and FR 77 08053 [O] are relied upon to demonstrate a large variety of cell separation devices available in the prior art and suitable for cell separation in the present invention directed to a method for isolating nucleated fetal cells from maternal peripheral blood intended for prenatal genetic investigation. The devices of the cited patents comprise an elongated chamber and channel(s) that are open to the chamber.

Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to use a large variety of cell separation devices suitable for separating blood cells including isolating nucleated fetal cells from maternal peripheral blood intended for prenatal genetic investigation as demonstrated by the cited references. The cited references are in the same field of endeavor and seek to solve the same problems as the instant application and claims such as blood cell separation, and one of skill in the art is free to select devices available in the prior art. Thus, the claimed invention as a whole was clearly prima facie obvious, especially in the absence of evidence to the contrary. Moreover, the devices disclosed by the cited patents US 4,424,132 [D], GB 2-75376 [N] and FR 77 08053 [O] are admitted by applicant as suitable in the presently claimed invention (specification page 6, par. 3). Thus, whatever differences might exist between various cell separation devices of the prior art and the particular device of the present invention, the claimed subject matter fails to patentably distinguish over the state art as represented by the cited references. Therefore, the claims are properly rejected under 35 USC § 103.

***Response to Arguments***

Applicant's arguments filed 6/30/2004 have been fully considered but they are not persuasive.

Applicant's main argument is directed to the idea that the presently claimed invention is patentably distinct from the cited prior art on the basis on the pH range of the tissue culture mixture (see page 15 of the response and Declaration under 37 CFR 1. 132 by Sitar, particularly page 3) and that prior art does not recognize pH value as result effective variable. This, argument is moot in view of the new ground(s) of rejection as applied to new claims 11-21 and 24.

Applicant also argues that the use of specific pH allows to separate maternal blood cells in "a single separation device" and/or in "a single centrifugation step". This argument is not found convincing since it is not particularly clear as claimed when and what cell fractions are separated from each other in "a single separation device" and/or "a single centrifugation step" and/or as intended.

With regard to the cited prior art applicant argues that centrifugation step in the methods of the cited reference is an initial step that results in separation of a mononuclear cell fraction {comprising fetal nucleated red blood cells and maternal monocytes and lymphocytes} and that the prior art methods require some additional steps for "true" separation of fetal cells from maternal monocytes and lymphocytes, for example: by FACS, by differential hemolysis or by DNA analysis (see response pages 16-17). Yet, the claimed method does not result in the production of a pure fraction of fetal cells that are fully separated/isolated from maternal cells as result of one and only centrifugation step. The claimed method comprises step of centrifugation and the other step of "ascertaining the presence of fetal NRBCs" and, thus, the claimed method comprises both initial and additional steps in contradiction to the argument(s). Therefore, the cited prior art, that demonstrates the presence of fetal cells in a mononuclear cell fraction obtained by centrifugation, teaches the same concept of isolation/separation of fetal cells by centrifugation. The prior art additional step(s) encompass(es) "ascertaining the presence of fetal NRBCs" such as subjecting cells to DNA analysis or cell sorting using antibodies and FACS within the meaning of the instant claims. Moreover, the applicant's method as disclosed demonstrates that centrifugation step results in a possession of a cell fraction that contains fetal nucleated red blood cells (NRBCs) together with maternal nucleated lymphocytes and

monocytes (specification page 10, lines 16-19; page 4, line 12). Thus, the applicant's method as disclosed is not a single step of centrifugation that would separate fetal nucleated cells from all maternal mononuclear cells.

Contents of the Declaration under 37 CFR 1.132 by Giammaria Sitar filed 6/30/2004 and a corrected draft proposed during interview on 9/15/2004 were fully considered. Applicant argues that the specific pH allows to separate fetal red blood nucleated cells from maternal nucleated cells. However, the instant invention as-filed appears to disclose separation of mononuclear cell fraction comprising fetal red blood nucleated and maternal nucleated cells from maternal red cells, for example: see original claim 1 and specification page 10, line 19. The fetal cells are identified (differentially stained and counted, see specification page 10, line 30) within the fraction of mononuclear cells comprising fetal red blood nucleated and maternal nucleated cells. No pure product of fetal cells is obtained as disclosed. Thus, the argument drawn to the use of specific pH does not appear to be relevant for the instant invention as argued.

No claims are allowed.

***Conclusion***

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period

will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Vera Afremova whose telephone number is (703) 308-9351 till January 15, 2004 or (571) 271-0914 after January 15, 2004. The examiner can normally be reached on 9.30 am - 6.00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached on (703) 308-4743 till January 15, 2004 or on (571) 272-0926 after January 15, 2004.

The fax phone number for the TC 1600 where this application or proceeding is assigned is (703) 872-9306.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Vera Afremova

AU 1651

November 19, 2003.

V. Afremova

VERA AFREMOVA

PATENT EXAMINER